

Host acceptance by three native braconid parasitoid species attacking larvae of the Mexican fruit fly, *Anastrepha ludens* (Diptera, Tephritidae)

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Abstract

We studied the oviposition and host acceptance behavior of three braconid parasitoid species native to Mexico, *Doryctobracon crawfordi* (Viereck), *Opius hirtus* (Fischer), and *Utetes anastrephae* (Viereck), with potential to be considered as biocontrol agents against tephritid fruit fly pests in the Neotropics. Third instar larvae of *Anastrepha ludens* (Loew), with and without previous parasitization by conspecifics, were simultaneously offered to females of each species, and the individual behavior was video recorded to construct oviposition flow diagrams. The patterns of foraging and host acceptance were similar in the studied species; all rejected mostly parasitized hosts suggesting that this strategy is common in the guild of larval parasitoids attacking *Anastrepha* spp. The complete searching and host acceptance process took 2.2 ± 0.1 min (mean \pm SE) in *D. crawfordi*, 1.7 ± 0.1 s in *U. anastrephae* and 1.5 ± 0.1 s in *O. hirtus*. Notably, because of toxins injected by parasitoid females during oviposition, the parasitized hosts experienced a transient paralysis of variable duration. Hosts attacked by *U. anastrephae* remained immobile for the shortest time (12.5 ± 1 min) (mean \pm SE), followed by *D. crawfordi* (20.5 ± 3.4 min) and *O. hirtus* (24.1 ± 2 min). Our data revealed a notable discrimination ability in all three species, and that behavioral differences lay

mainly in the time of parasitization and in the duration of paralysis experienced by attacked hosts. This suggest that the three species could be valuable as biocontrol agents, but additional studies are necessary to better understand the advantages and limitations of each one as natural enemies of fruit fly pests.

Keywords

Host discrimination, transient host paralysis, biocontrol agents, *Doryctobracon crawfordi*, *Opius hirtus*, *Utetes anastrephae*

Introduction

Fruit flies (Diptera: Tephritidae) are considered one of the main fruit pests worldwide (Enkerlin 2005). To reduce pest populations, various control tactics have been developed among which the augmentative release of parasitoids has arisen as one sound and well oriented strategy against these pests (Sivinski et al. 1996, Montoya et al. 2007).

Parasitoids are insects whose larvae develop by feeding in or on the body of other arthropods, usually insects; larval feeding almost always results in the death of the host (Godfray 1994). Parasitoids are immersed in a multitrophic context (Hassell and Waage 1984, Vet and Dicke 1992), where foraging for nutrients and hosts is performed at different scales (Kramer 2001, Gingras et al. 2002). In general, it is the female parasitoid that locates a suitable host. Because parasitoid development is dependent on limited resources (the host), adult preference and larval performance should be correlated to maximize fitness (Harvey et al. 2015) and the host acceptance procedure is considered the definitive step in host searching behavior (Vinson 1984). The hosts are often hidden in the interior of stems, leaves or fruits (Richerson and Borden 1972), consequently parasitoid females must detect and respond to a number of indirect signals where chemical-sensorial information plays a fundamental role (Vinson 1976, 1998, van Alphen and Vet 1986, Vet and Dicke 1992). In addition to chemical stimuli, parasitoids are also capable of identifying vibrations emitted by their hosts through the substrates in which they develop (van Alphen and Janssen 1982, Vet and van Alphen 1985, Meyhöfer et al. 1997).

Once female parasitoids have located their hosts, they have the capacity to distinguish between parasitized and not parasitized hosts, a strategy known as discrimination ability (van Alphen and Visser 1990). This ability can occur at three levels: (1) self-discrimination, (2) conspecific discrimination and (3) heterospecific discrimination (Mackauer 1990). This ability has been observed in many species of hymenoptera parasitoids (Vinson 1976), and is particularly important in the case of potential biocontrol agents, since these are expected to be efficient in host searching and to have the ability to discriminate between parasitized and non-parasitized hosts (van Lenteren et al. 1978). The latter helps females to avoid superparasitism, reducing the time and energy spent in searching behavior (Mackauer 1990, Godfray 1994).

Host location and host acceptance behavior has been widely studied in the generalist fruit fly parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Greany et al. 1977,

Lawrence 1981, Carrasco et al. 2005). This species is exotic in the Americas where it has been successfully reared for augmentative biological control of *Anastrepha* (Schiner) fruit flies in Mexico (Montoya et al. 2000, 2007) and in Florida USA (Sivinski et al. 1996); and for *Ceratitis capitata* (Wiedemann) in Argentina (Sanchez et al. 2016). However, there is a guild of native opiine braconid parasitoids (Sivinski et al. 2000, 2001) with potential as biocontrol agents, for which little information exists regarding their foraging and host acceptance behavior. This is the case of *Doryctobracon crawfordi* (Viereck), *Utetes anastrephae* (Viereck) and *Opius hirtus* (Fischer), all of which are solitary, larval-pupal endoparasitoids of *Anastrepha* spp. (López et al. 1999), that coexist in different regions of America (Sivinski et al. 2000). It has been postulated that differences in ovipositor size, as well as specific foraging behaviors, serve to prevent direct competition among these species (Ovruski et al. 2000, Sivinski et al. 2000). Under laboratory conditions, the three species can develop in the third instar larvae of *Anastrepha ludens* (Diptera: Tephritidae) (Aluja et al. 2009).

Doryctobracon crawfordi is native in habitats above 600 masl from Mexico to Argentina (Ovruski et al. 2005); possess a long ovipositor 5.39 ± 0.08 mm and attacks *Anastrepha* spp. mainly in citrus fruits and is sensitive to both high temperature and low humidity (Sivinski et al. 2000). *Utetes anastrephae* is characterized by a short ovipositor (1.57 ± 0.04 mm, Sivinski et al. 1997, 2001) and can be found associated with small fruits such as those of *Spondias* spp. (Anacardiaceae), with 2-5 cm of diam and 4 to 33 g weight (Avitia 2000). *Opius hirtus* is a more specialized parasitoid being recovered from *Anastrepha obliqua* (Macquart) in *Spondias mombin* L. and from *Anastrepha alveata* (Stone) infesting *Ximenia americana* L. (Olacaceae) (Sivinski et al. 2000). The three species are synovigenic (Sivinski et al. 2001).

The purpose of this study was to compare the foraging and host acceptance behaviors of the parasitoid species *D. crawfordi*, *U. anastrephae* and *O. hirtus* on previously parasitized and non-parasitized larvae of *A. ludens*, using video recording equipment under laboratory conditions. This knowledge should allow an improved understanding of the oviposition performance and potential of these parasitoid species as biocontrol agents against fruit fly pest species.

Material and methods

Study site and biological material

The experiments were conducted in the Biological Control laboratory of the Moscafrut Program SAGARPA-IICA, located in Metapa de Dominguez, Chiapas, Mexico. The parasitoid colonies were initiated from field infested fruits and maintained at 25 ± 1 °C, $70 \pm 5\%$ HR with a photoperiod of 12:12 (L:D) h. Eight-day-old larvae of *A. ludens* mixed with artificial diet were provided as host by the Moscafrut facility, where this species is mass reared as described by Orozco-Dávila et al. (2017). Adult parasitoids of the species *D. crawfordi*, *U. anastrephae* and *O. hirtus* were reared according to Aluja et al. (2009).

Preparation of host larvae

Parasitized host larvae were obtained by exposing groups of approximately 100 host larvae for two hours to 100 females and 50 males of each species separately. Larvae with three or more oviposition scars were considered as being successfully parasitized (Montoya et al. 2000, 2003). Host larvae without previous parasitization were allocated to the “not parasitized host group”.

Preparation of the parasitoids

Copulated females, 5–6 day old with previous experience of oviposition were used. To gain this experience, groups of ~150 recently emerged adults (1female: 1male) were confined in aluminum frame acrylic cages (30 × 30 × 30 cm) and provided with water and honey as a source of food. Twenty-four hours before conducting the bioassays, ~200 *A. ludens* larvae mixed with larval diet were offered to these parasitoids in a Petri dish oviposition unit, for 2 h.

Host acceptance test

The host searching and acceptance performance of individual parasitoid females was observed with two different types of *A. ludens* host larvae that were exposed simultaneously: 1) larvae previously parasitized (24 h earlier) by conspecifics, and 2) larvae with no previous parasitization. Bioassays were conducted in oviposition units consisting of Petri dishes (55 mm in diameter × 9 mm in depth) with the edges reduced to five mm in depth and a central division of 5 mm to separate the two type of larvae. Five previously parasitized *A. ludens* larvae were placed in one of the two sides, and five non-parasitized larvae, of the same age, were placed in the other side. The oviposition unit was covered with an organza elastic cloth and secured with a rubber band in order to prevent larval escape. This cloth was semi-transparent making possible the observation of the host larvae through it. Guava juice was added on the surface of the cloth in order to attract the females and keep them on the parasitization units until larval detection.

Video recording procedure

The oviposition sequences of thirty females per species were observed and video recordings made with a Samsung KREUZNACH video camera (f = 2.3–78.2 mm; F:1.6; ø30.5). One female was released onto the surface of the oviposition unit in each observation. The larvae and females were replaced after each observation, as well as the cloth and the oviposition unit. Environmental conditions were 25 ± 1 °C and 75 ± 5%

RH. Bioassays were conducted between 8:30 and 15:00 and the time of observation was ~1 h per female. If the female presented null activity for the first five minutes, it was replaced. Time of latency (defined here as “time that elapsed between two ovipositions”), the number of ovipositions, oviposition attempts, duration of oviposition and duration of host paralysis following oviposition (from the moment the stung larva remained immobile, to the moment it resumed crawling), were recorded for both host types. Video recordings were independently analyzed using the *Movie Maker* software version 2.6.4037.0, in order to obtain the sequences and transition frequencies of the different behaviors.

Statistical analysis

The number of ovipositions and oviposition attempts on the two larval types were compared using the *t* test for each parasitoid species. In order to compare the time spent on the different activities observed among the three species, a one-way analysis of variance with the Tukey-HSD test was conducted. Prior to analysis, a Box -Cox transformation of the data was conducted. For all analyses we used the JMP Starter software version 7.0.1 (SAS Institute 2007).

Results

The general behavioral sequences of the three parasitoid species on the two host types were identified. The operational definitions for the observed behaviors are presented in Table 1. The most common sequence for any of the three-braconid species included: 1. Walking (W), 2. Searching for a host (S), 3. Detection of a host (D), 4. Oviposition attempt (OP), 5. Oviposition (O), 6. Rejection (RE), and 7. Failure (F) with some variants occurring depending on species (Figs 1a, b; 2a, b; 3a, b).

Searching and oviposition behavior

In general, the females walked on the surface of the oviposition unit with their antennae in close contact with the surface of the oviposition unit. Once the females detected a larva, they attempted to establish contact with the host by introducing their ovipositor and began a movement of abdominal vibration (associated with the descent of the egg (Montoya et al. 2009); they then moved the antenna and extracted the ovipositor. Even though the three species maintained a similar pattern of oviposition behavior, *U. anastrephae* was often observed to perform a wing movement when inserting its ovipositor into a host. *D. crawfordi* rotated on its axis once contact was established with the larva. These specific behavioral acts led to successful ovipositions (Figs 1, 2 and 3).

Table 1. Definitions of the different behaviors exhibited by *Utetes anastrephae*, *Doryctobracon crawfordi* and *Opius hirtus* while foraging for host larvae.

Behavior	Description
1. Walking	Female walking on the oviposition unit surface, antennae not directed to the substrate
2. Searching for a host	While walking the female touches the surface of the oviposition unit with the antennae
3. Detection of a host	The female stays immobile over a host larva
4. Oviposition attempt	Insertion of the ovipositor in order to have contact with the host. The latter is very mobile
5. Oviposition	Oviposition, the female remains immobile during a certain period of time with the ovipositor inserted in the interior of the host larva
6. Rejection	The female inserts the ovipositor in the host for a few seconds, but withdraws the ovipositor without actually laying an egg.
7. Failure	When the female inserts the ovipositor in the oviposition unit without having contact with some host, mainly by the escape of the larvae

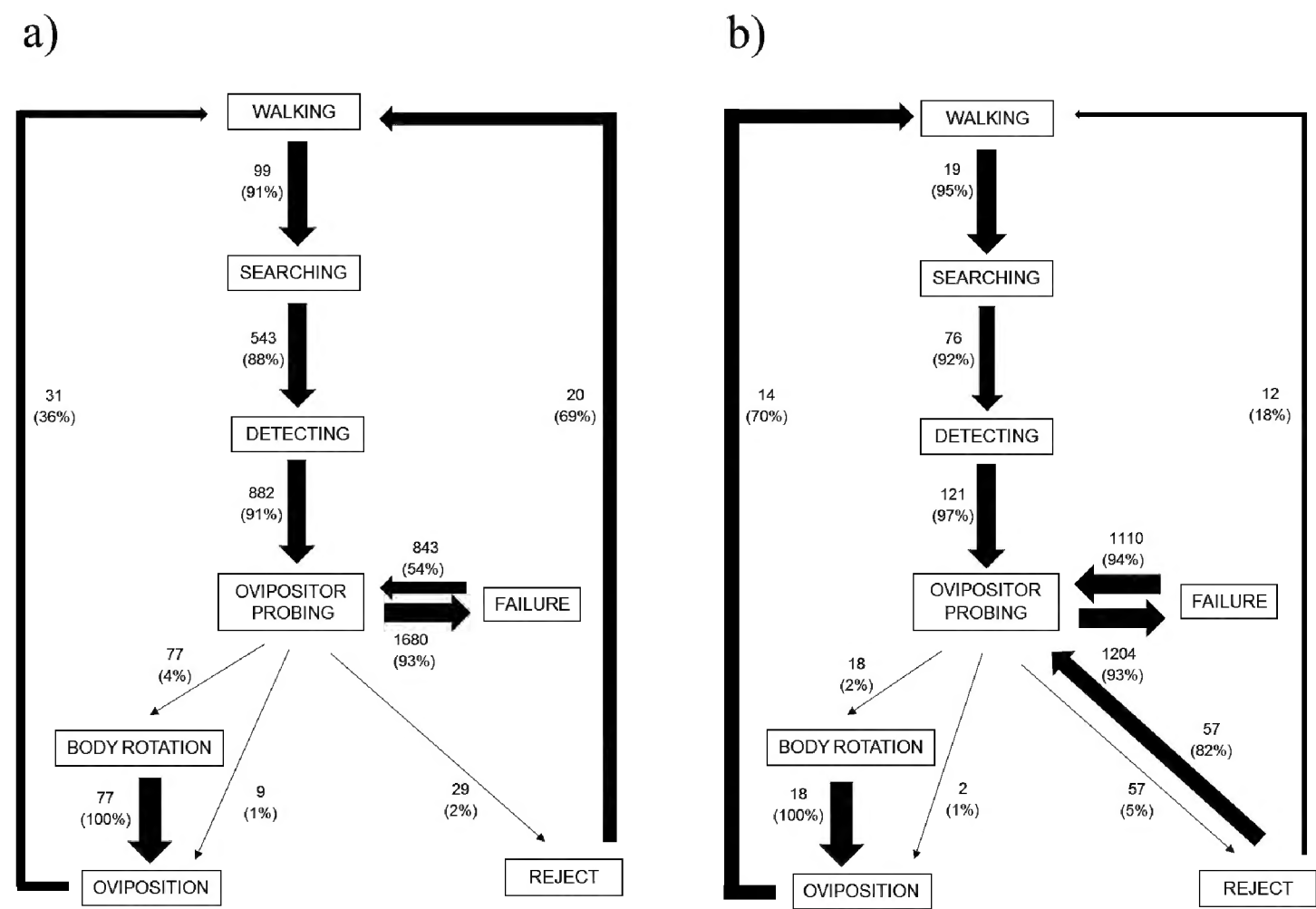


Figure 1. Ethogram of oviposition of females of *Doryctobracon crawfordi* on non-parasitized larvae (**a**) and larvae previously parasitized by conspecifics (**b**) under laboratory conditions. The width of the arrow is proportional to the relative frequency of transition. The numbers associated with the arrows represent the observed frequencies of the successive behaviors of a complex sequence of behavior (proportions are indicated in parentheses).

No marked differences in the flow diagrams were observed between non-parasitized hosts and parasitized hosts for any of the braconids studied here. However, females significantly rejected hosts previously parasitized by conspecifics following insertion of the

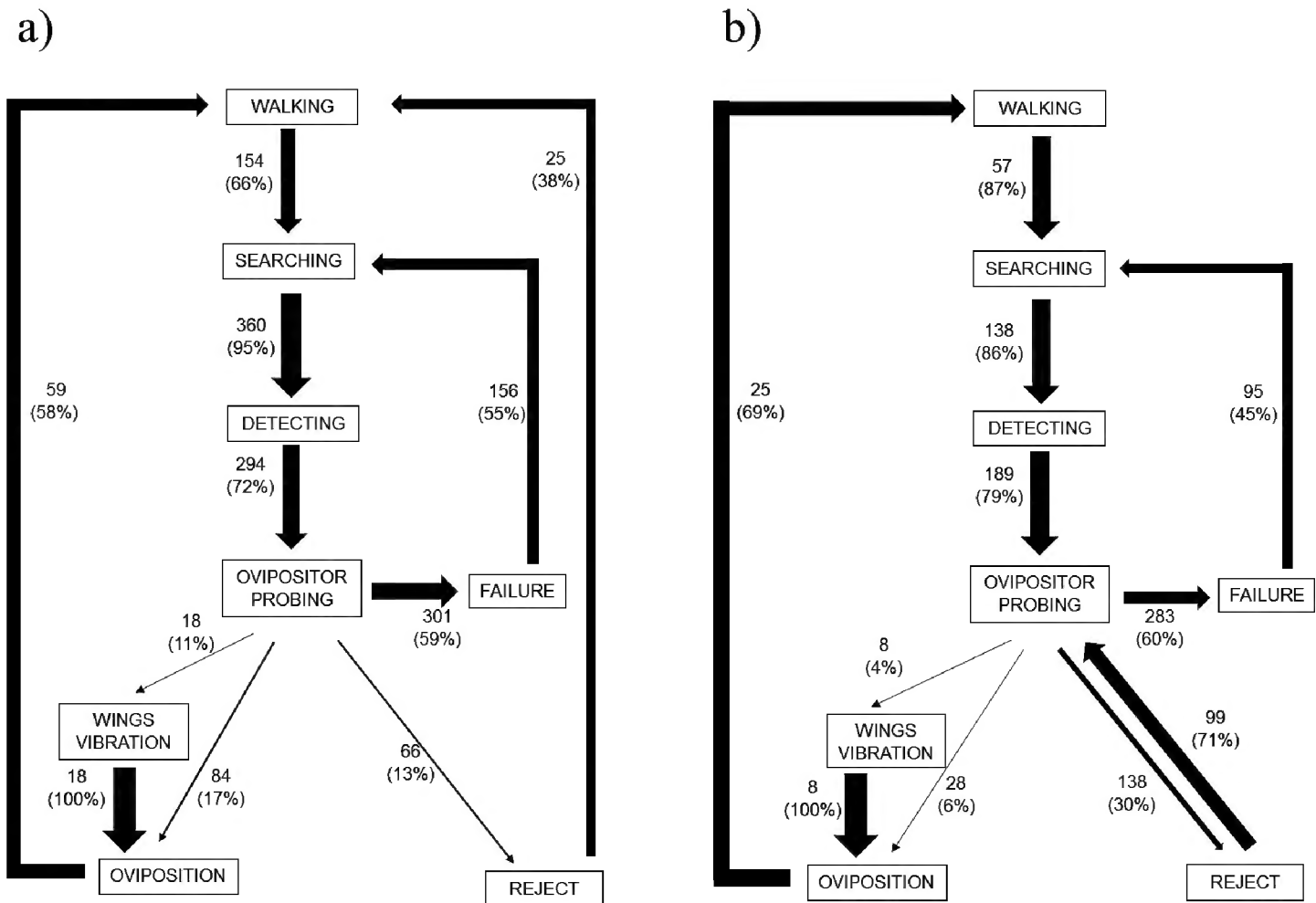


Figure 2. Ethogram of oviposition of females of *Utetes anastrephae* on non-parasitized larvae (a) and larvae previously parasitized by conspecifics (b) under laboratory conditions. The width of the arrow is proportional to the relative frequency of transition. The numbers associated with the arrows represent the observed frequencies of the successive behaviors of a complex sequence of behavior (proportions are indicated in parentheses).

ovipositor compared to those not parasitized ($F = 2.35$; $df = 2$, $P < 0.001$). Overall, *U. anastrephae* females rejected 79% of parasitized hosts, *D. crawfordi* 74% and *O. hirtus* 62%. Furthermore, a more intensive searching was observed when a failure (because the host moved away) occurred when attacking non-parasitized hosts than when attacking parasitized hosts. The complete process of searching and host acceptance (from the beginning of the observation until ovipositor removal) was completed in 2.2 ± 0.8 min (mean \pm SE) in *D. crawfordi*, 1.7 ± 0.75 min in *U. anastrephae* and 1.52 ± 0.75 min in *O. hirtus*.

Latency

The time elapsed between ovipositions differed significantly between *U. anastrephae* and the other two species when the hosts had previously been parasitized ($F = 0.5$, $df = 2$, $P < 0.05$; $N = 30$). Regarding the time of latency with non-parasitized larvae, *U. anastrephae* presented the shortest time (3.25 ± 0.3 min) (mean \pm SE) (Fig. 4) ($F = 10.6$, $df = 2$, $P < 0.001$), compared to that of the other two species (*D. crawfordi* = 4.88 ± 0.48 min and *O. hirtus* = 5.65 ± 0.75 min) (mean \pm SE).

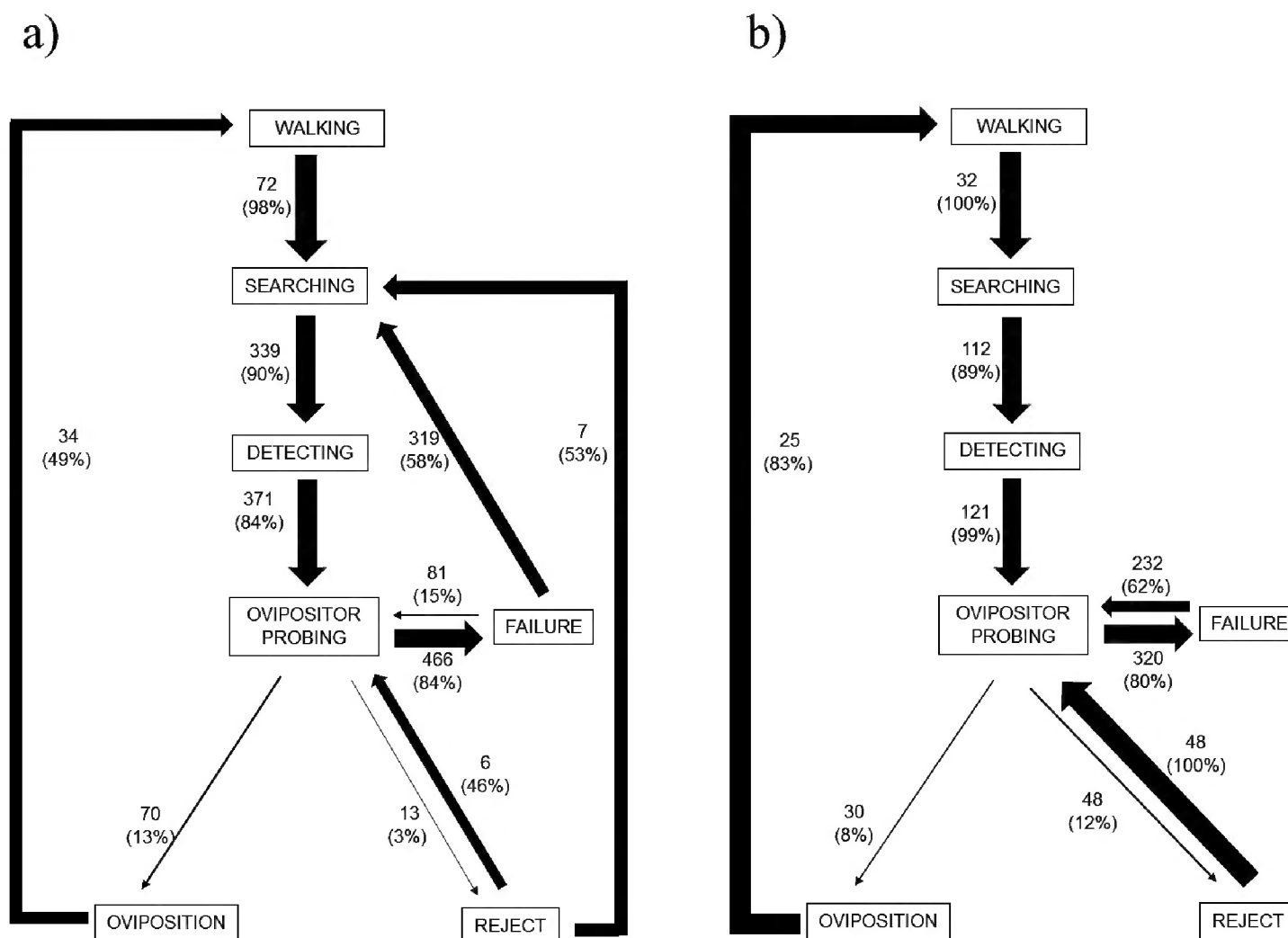


Figure 3. Ethogram of oviposition of females of *Opius hirtus* on non-parasitized larvae (a) and larvae previously parasitized by conspecifics (b) under laboratory conditions. The width of the arrow is proportional to the relative frequency of transition. The numbers associated with the arrows represent the observed frequencies of the successive behaviors of a complex sequence of behavior (proportions are indicated in parentheses).

Discrimination ability

The first host choice in the three parasitoid species corresponded mostly to the non-parasitized larvae (*D. crawfordi* 22/30; *U. anastrephae* 18/30 and *O. hirtus* 19/30). *Utetes anastrephae* parasitized a significantly ($F = 3.39$, $df = 2$, $P = 0.03$) higher quantity of non-parasitized hosts (3.3 ± 0.25) compared to the other two species (*D. crawfordi* 2.7 ± 0.23 and *O. hirtus* 2.3 ± 0.32). *Doryctobracon crawfordi* performed a greater number of oviposition attempts than *U. anastrephae* and *O. hirtus* in both types of larvae (Table 2).

The time of ovipositor insertion on previously parasitized larvae differed significantly ($F = 4.7$, $df = 2$, $P = 0.001$) among species, with *D. crawfordi* spending more time with the ovipositor inserted, and *O. hirtus* the shortest one (Table 3). The time of ovipositor insertion of *O. hirtus* was significantly shorter in previously parasitized larvae compared to that in non-parasitized larvae ($t = 2.67$, $df = 67$, $P = 0.0094$, Table 3), while in the other two species no significant difference was found between the two host types. No significant differences were found between the two types of larvae in the duration of abdomen vibration and duration of the host paralysis after the attack by each parasitoid species. However, the duration of paralysis of the host differed among

Table 2. Average values (\pm SE) of number of ovipositions and attempts at oviposition on host larvae parasitized by conspecifics and non-parasitized host larvae.

Species of parasitoid	Number of ovipositions		Number of oviposition attempts (rejections)		N
	Non-parasitized larvae	Parasitized larvae	Non-parasitized larvae	Parasitized larvae	
<i>Doryctobracon crawfordi</i>	2.7 \pm 0.23 ^{ab}	0.6 \pm 0.15*	56.9 \pm 5.1 ^a	42.4 \pm 7.5 ^a	30
<i>Utetes anastrephae</i>	3.3 \pm 0.25 ^a	1.3 \pm 0.23*	12.2 \pm 2.1 ^b	14.7 \pm 2.2 ^b	30
<i>Opius hirtus</i>	2.3 \pm 0.32 ^b	1 \pm 0.16*	15.9 \pm 2.2 ^b	12.26 \pm 2.3 ^b	30

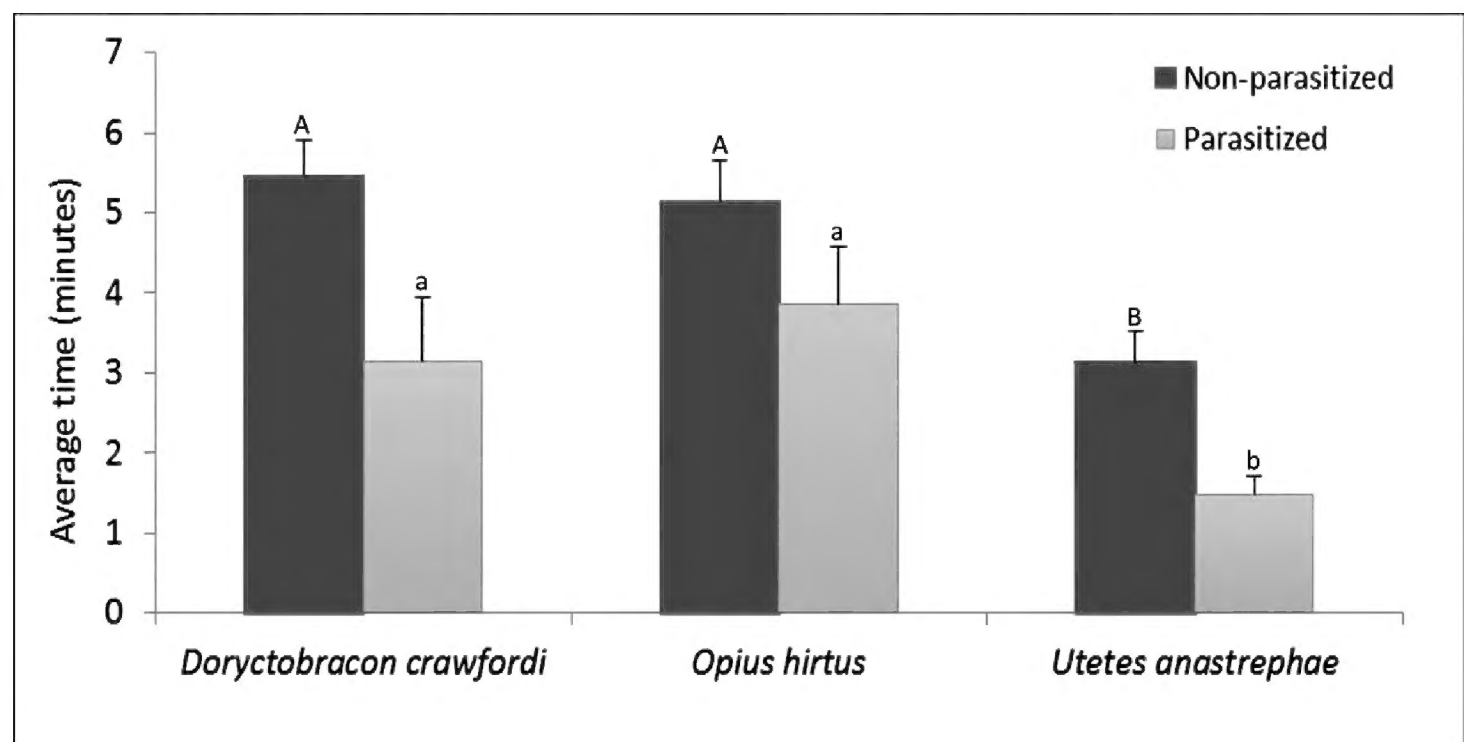
Different letters indicate statistically significant differences (Anova, $\alpha=0.05$) between species of parasitoids.

*Indicates a statistically significant difference between parasitized and non-parasitized hosts.

Table 3. Average values (\pm SE) of duration of oviposition, vibration of the abdomen of the females, and immobility of the host after stinging (all in minutes) in non-parasitized and parasitized host larvae of *A. ludens*.

Species	Duration of oviposition		Vibration of the abdomen		Host immobility		N
	Unparasitized host	Parasitized host	Unparasitized host	Parasitized host	Unparasitized host	Parasitized host	
<i>Doryctobracon crawfordi</i>	2.2 \pm 0.1 ^a	2.2 \pm 0.1	0.35 \pm 0.01 ^a	0.35 \pm 0.03	21.3 \pm 1.2 ^a	20.5 \pm 3.4	30
<i>Utetes anastrephae</i>	1.6 \pm 0.1 ^b	1.9 \pm 0.1	0.28 \pm 0.01 ^{ab}	0.26 \pm 0.01	13.4 \pm 0.6 ^b	12.1 \pm 1	30
<i>Opius hirtus</i>	1.5 \pm 0.1 ^b	1.2 \pm 0.1*	0.26 \pm 0.01 ^b	0.3 \pm 0.01	23.8 \pm 1.2 ^a	24.5 \pm 2	30

Different letters indicate a statistically significant difference per columns for each parameter (ANOVA, $\alpha=0.05$). *Indicates a statistically significant difference between parasitized and non-parasitized hosts for each species per parameter.

**Figure 4.** Latency (average \pm SE, in minutes) between ovipositions of three native opine parasitoids attacking non-parasitized and previously parasitized *Anastrepha ludens* larvae. Different capital letters indicate statistically significant difference between the bars. Different letters, indicate statistically significant difference between the bars. Different lower case letters, indicate statistically significant difference between species.

the three species (parasitized hosts, $F = 12.8$, $df = 2$, $P = 0.00001$; non-parasitized hosts, $F = 29.5$, $df = 2$, $P = 0.000001$). All hosts successfully stung showed temporary paralysis: *D. crawfordi* = 21.3 ± 1.2 min, *O. hirtus* = 23.8 ± 1.2 min and *U. anastrephae* = 13.4 ± 0.6 min) (mean \pm SE) (Table 3).

Discussion

Knowledge on host acceptance behavior in insect parasitoids is fundamental to improve our understanding on the plant-herbivore-natural enemy tritrophic relations (Vet and Dicke 1992), as well as the population dynamics and their possible implications in pest biological control programs (Minkenberg et al. 1992).

Several studies have indicated that responses of natural enemies are mediated mainly by chemical signals detected in the environment (Vinson 1998, Vet and Dicke 1992, van Alphen and Visser 1990), by host-generated vibrations in its microhabitat (Meyhöfer et al. 1997, Vet and van Alphen 1985), and by the individual learning experiences of foraging females. It is stated that parasitoids perceive stimuli about host quality once direct contact has been made with the host, influencing the host acceptance process (Brodeur and Boivin 2004, Wajnberg et al. 2008). Here we established the patterns of host acceptance by *D. crawfordi*, *U. anastrephae* and *O. hirtus* in the presence of both non-parasitized and previously parasitized hosts. We further characterized the time spent in different behaviors, and the duration of the paralysis induced through parasitism.

The three studied species presented typical behavior of antennal contact with the surface of the oviposition unit during the process of searching for the host larvae, which is an important step for host detection (Leyva et al. 1991, Gonzalez et al. 2010). All of the species presented a similar foraging pattern, beginning the search for the host by walking and touching the oviposition surface with the antennae. Once a larva was detected, the females adopted an alert position that consisted of remaining immobile for some seconds with the antennae extended to the front, skimming the surface of the parasitization unit. On locating a larva, the females performed small turns on their axis until positioning their first pair of legs towards the front and arranging their ovipositor to form a 90° angle to the contact surface. On initiating the process of oviposition in the parasitization unit, as reported for *D. longicaudata* (Montoya et al. 2003), the females make various attempts to insert the ovipositor until contact was made with a host, which then was accepted or rejected.

According to our results, the three parasitoid species have a high discrimination ability in the form defined by van Alphen and Visser (1990), given that females rejected most of the previously parasitized hosts compared to those with no previous parasitization. However, *U. anastrephae* notoriously presented the highest frequency of rejection of parasitized hosts (79%). This suggests that this species possesses a high performance avoiding superparasitism and saving time and energy when foraging for their hosts (Godfray 1994, Mackauer 1990). This corroborates previous findings by Aluja et

al. (2013), who showed that this species avoids ovipositing on previously parasitized hosts under conspecific and heterospecific situations, although it also has been noted that superparasitized hosts yielded relatively more daughters (Alvarenga et al. 2016), as referred for *D. longicaudata* (Montoya et al. 2011). The host acceptance behavior presented by the three species was similar to that reported for *D. longicaudata* by Montoya et al. (2003), who observed that the previously parasitized hosts experienced a lower number of ovipositions than the hosts with no previous parasitization.

Doryctobracon crawfordi presented the longest time spent on oviposition compared to the other two species. Host acceptance may depend on extrinsic factors such as host availability and quality, as well as intrinsic factors such as the quantity of eggs in the females, the age and their nutritional state (Vet et al. 2002, Bernstein and Jervis 2008). In the case of *D. crawfordi*, availability of eggs in the females can be an important limiting factor (Iwasa et al. 1984). Females of this species may tend to be more selective, avoiding oviposition on previously parasitized hosts or those considered to be of poor quality (Rosenheim 1996, Ayala et al. 2014). This could explain the large numbers of oviposition attempts (host probing) observed and the greater time on selection of non-parasitized hosts. The native parasitoids *D. crawfordi*, *U. anastrephae* and *O. hirtus* invest more time in the process of oviposition (2.2 ± 0.8 , 1.7 ± 0.1 and 1.52 ± 0.75 min, respectively) than exotic species such as *D. longicaudata* (0.49 ± 0.2 min; Montoya et al. 2003) and *D. tryoni* (0.69 ± 0.065 min; Ramadan et al. 1994) under laboratory conditions. This could be related to the level of host discrimination ability, since *D. longicaudata* has a strong tendency to superparasitize (Montoya et al. 2003) while the native species here studied seem to avoid superparasitism. In *O. hirtus*, the duration of oviposition when parasitizing previously parasitized hosts was significantly smaller (1.2 ± 0.1 min) than with non-parasitized hosts (1.6 ± 0.1). The time invested in oviposition can vary according to the particular species and host size (Rivero 2000). In our study, females with experience that had contact with previously parasitized hosts, proved to be the most insistent and inserted their ovipositor a second time in order to conduct contact (*D. crawfordi* 16/30, *U. anastrephae* 22/30 and *O. hirtus* 16/30).

Though koinobionts do not arrest host development, some species can induce transient host paralysis (temporary paralysis after being stung by the female wasp; e.g. Desneux et al. 2009, Chau and Maeto 2009). Our data show that the three braconids studied here temporarily paralyzed their hosts, with 100 percent of hosts undergoing transient paralysis. Interestingly, the duration of paralysis was species specific. Larvae parasitized by *U. anastrephae* remained immobile for 13 ± 1 minutes, and thus presented this state for the shortest time, compared to the immobility presented by host larvae parasitized by *D. crawfordi* and *O. hirtus* (20.9 ± 1.1 and 23.8 ± 1 min, respectively). The duration of immobility caused by oviposition of the native parasitoids exceeds the time of immobility experienced by larvae parasitized by *D. longicaudata* (4.8 ± 27 min; Montoya et al. 2003).

The factors associated with host immobility are toxic substances in a mixture such as venom, as well as polydnviruses (PDVs) that function as regulatory elements and disrupt the host metabolism (Moreau and Guillot 2005, Kaeslin et al. 2010), affecting

the immune system (Richards and Parkinson 2000, Cai et al. 2004). Two hypothesis have been advanced to explain the adaptive value of transient host paralysis: 1) facilitation of oviposition by interfering with host defensive behaviors; and 2) self-superparasitism avoidance. Support for the latter hypothesis comes from the work of Desneux et al. (2009) with two species of aphidiine braconids of the genus *Binodoxys* that attack aphids, and from the work of Chau and Maeto (2009) with *Meteorus pulchricornis* also a braconid (Euphorinae) that attacks a wide range of lepidopteran larvae. Transient paralysis caused by *Binodoxys* spp. lasted up to 15 min and paralyzed aphids were accepted at a significantly lower rate than control aphids (Desneux et al. 2009). Likewise, in *M. pulchricornis*, host movements remained at a low level for approximately 1h after oviposition, and additional ovipositions on paralyzed hosts were not observed (Chau and Maeto 2009). We further hypothesize that transient host paralysis may also be a means to avoid host detection by conspecifics and heterospecific competitors, reducing the risk of larval competition not only from superparasitism but also from multiparasitism. In solitary endoparasitoids only one adult emerges per host, all other larvae are eliminated through direct (intrinsic) competition. Intrinsic competition in the guild of opine braconids that attack *Anastrepha* spp. has been demonstrated in *U. anastrephae* and *D. areolatus*, with the first instar larva of *U. anastrephae* being a superior competitor (Aluja et al. 2013). The duration of paralysis of the host in the three species studied here may allow some advantage to the developing embryo, delaying additional attacks. The first eclosed first instar larva might have more chances to win when competing with second laid individuals.

There are few studies regarding the oviposition behavior of opiine parasitoid species native to the Neotropical region, which makes our data of valuable importance. Our study reveals that behavioral differences among the studied parasitoid species lay mainly in the time of parasitization and in the time for which the parasitized hosts remained immobile, which could delay or minimize superparasitism. The three species were significantly capable of discriminating previously parasitized hosts, suggesting that this strategy is commonly present in the guild of fruit fly parasitoids attacking larvae in the Neotropics. Finally, our data also suggest that the studied species have the potential to be considered as suitable biological control agents. However, more studies are necessary to better understand the advantages and limitations that each one presents as natural enemies of fruit fly pests under field conditions.

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